FIG.1

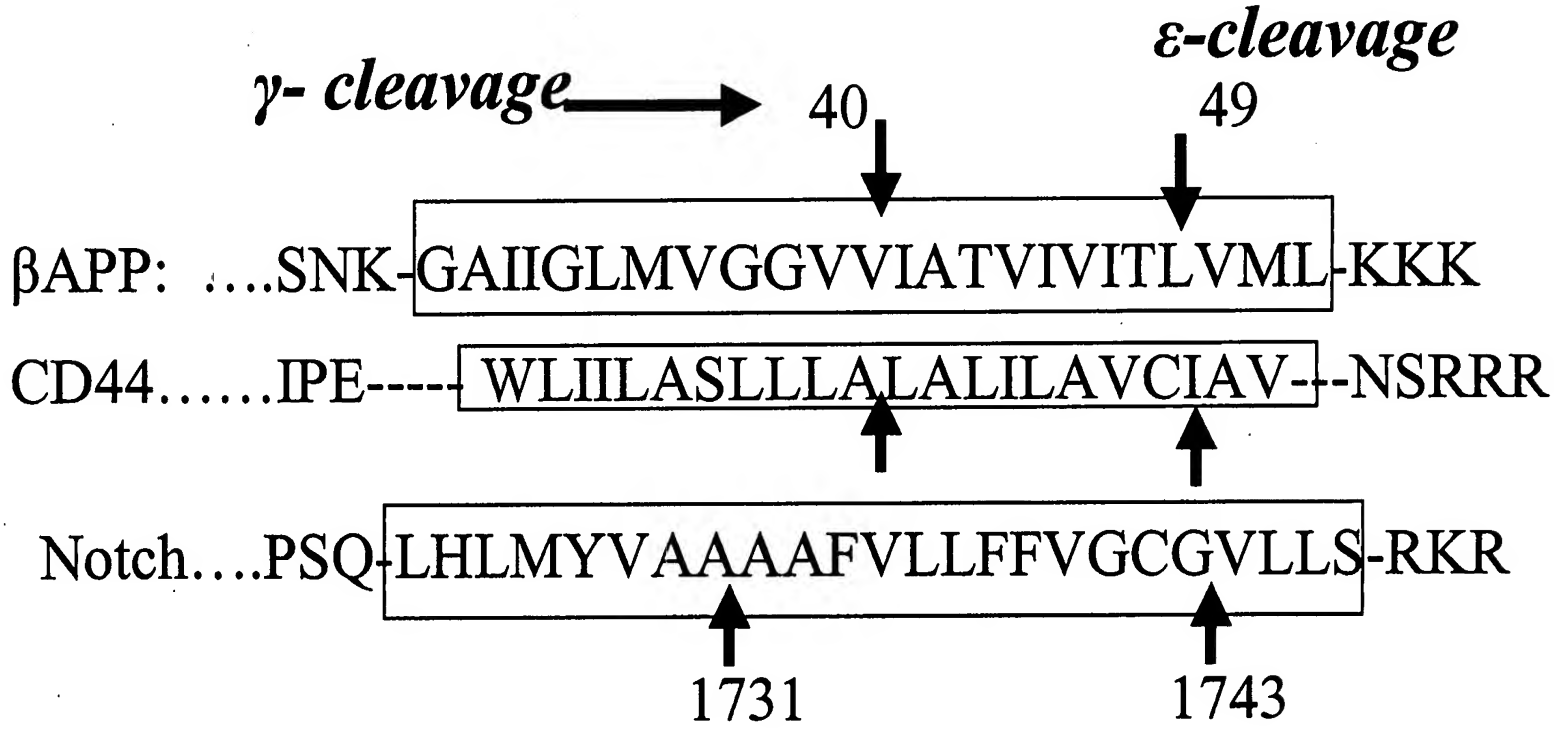
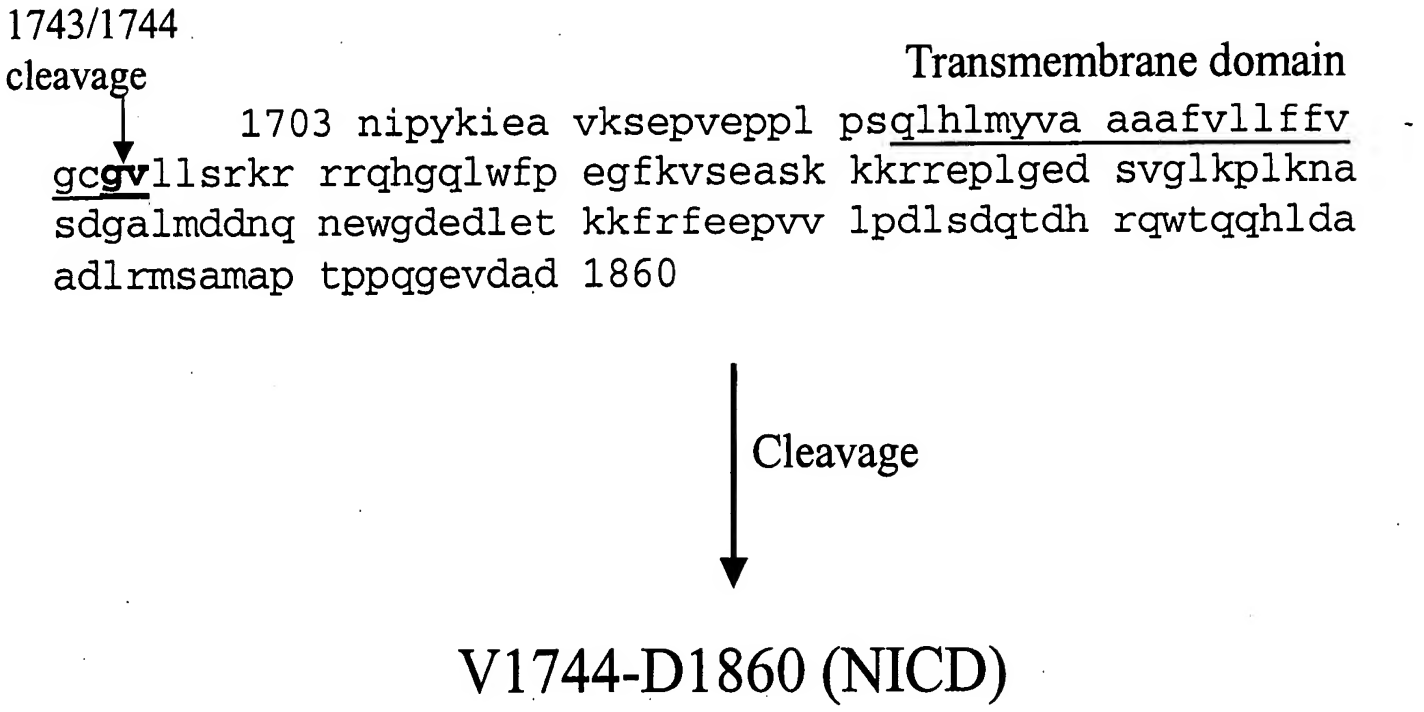
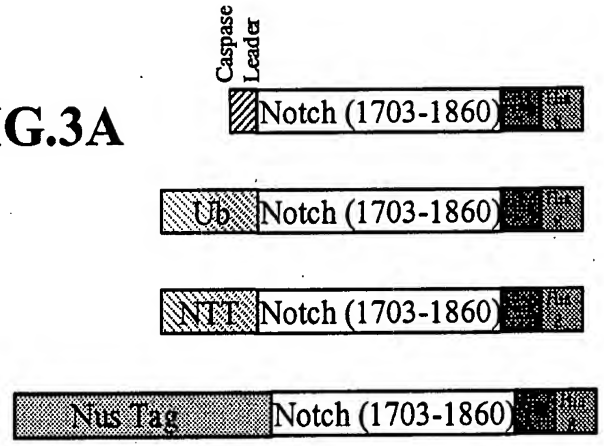

ε-cleavage
γ- cleavage
40
49
βAPP: ….SNK-GAIIGLMVGGVVIATVIVITLVML-KKK
CD44……IPE-----WLIILASLLLALALILAVCIAV--NSRRR
Notch….PSQ-LHLMYVAAAAFVLLFFVGCGVLLS-RKR
1731
1743

FIG. 2


1743/1744
cleavage
Transmembrane domain
1703 nipykiea vksepveppl psqlhlmyva aaafvllffv
gcgvllsrkr rrqhgqlwfp egfkvseask kkrreplged svglkplkna
sdgalmddnq newgdedlet kkfrfeepvv lpdlsdqtdh rqwtqqhlda
adlrmsamap tppqgevdad 1860
Cleavage
V1744-D1860 (NICD)

Constructs for Notch Expression

FIG.3A



No Expression

No Expression

No Expression

Soluble Expression

Cloning of Nus-Notch

FIG.3B

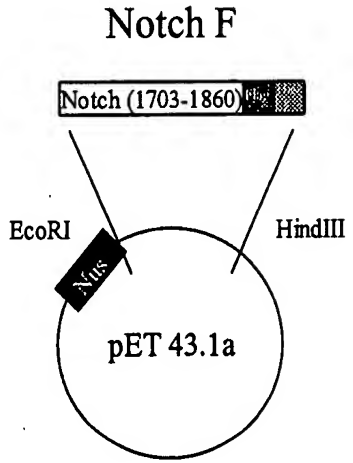


FIG. 4

Lane	Sample
1	Crude extract
2	Supernatant
3	IMAC flow through
4	50 mM imidazole wash
5-9	300 mM imidazole elution
10	MW markers

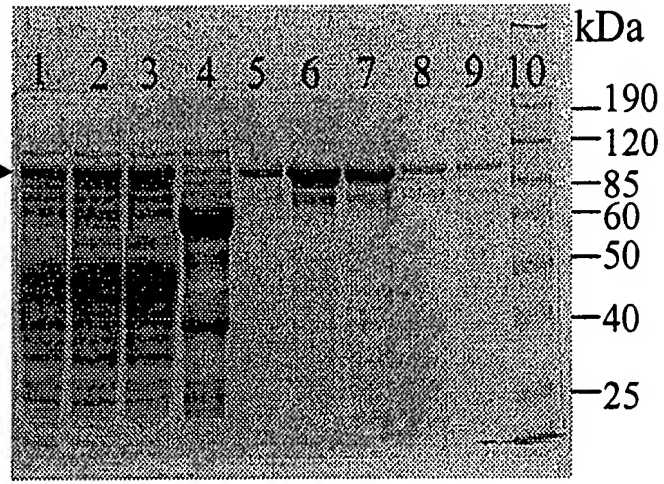


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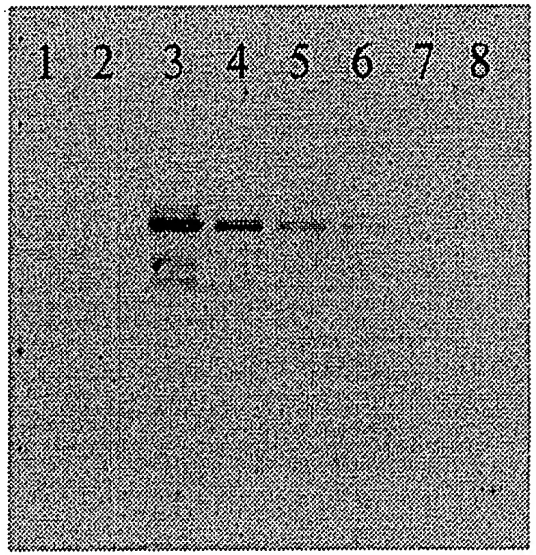
FIG. 5

Lane	Sample
1	Notch fusion (No γ-secretase)
2	γ-secretase (No substrate)
3	Notch + γ-secretase
4	Notch + γ-secretase + 2 nM DAPT
5	Notch + γ-secretase + 7.8 nM DAPT
6	Notch + γ-secretase + 31.2 nM DAPT
7	Notch + γ-secretase + 125 nM DAPT
8	Notch + γ-secretase + 500 nM DAPT



BEST AVAILABLE COPY

FIG. 6

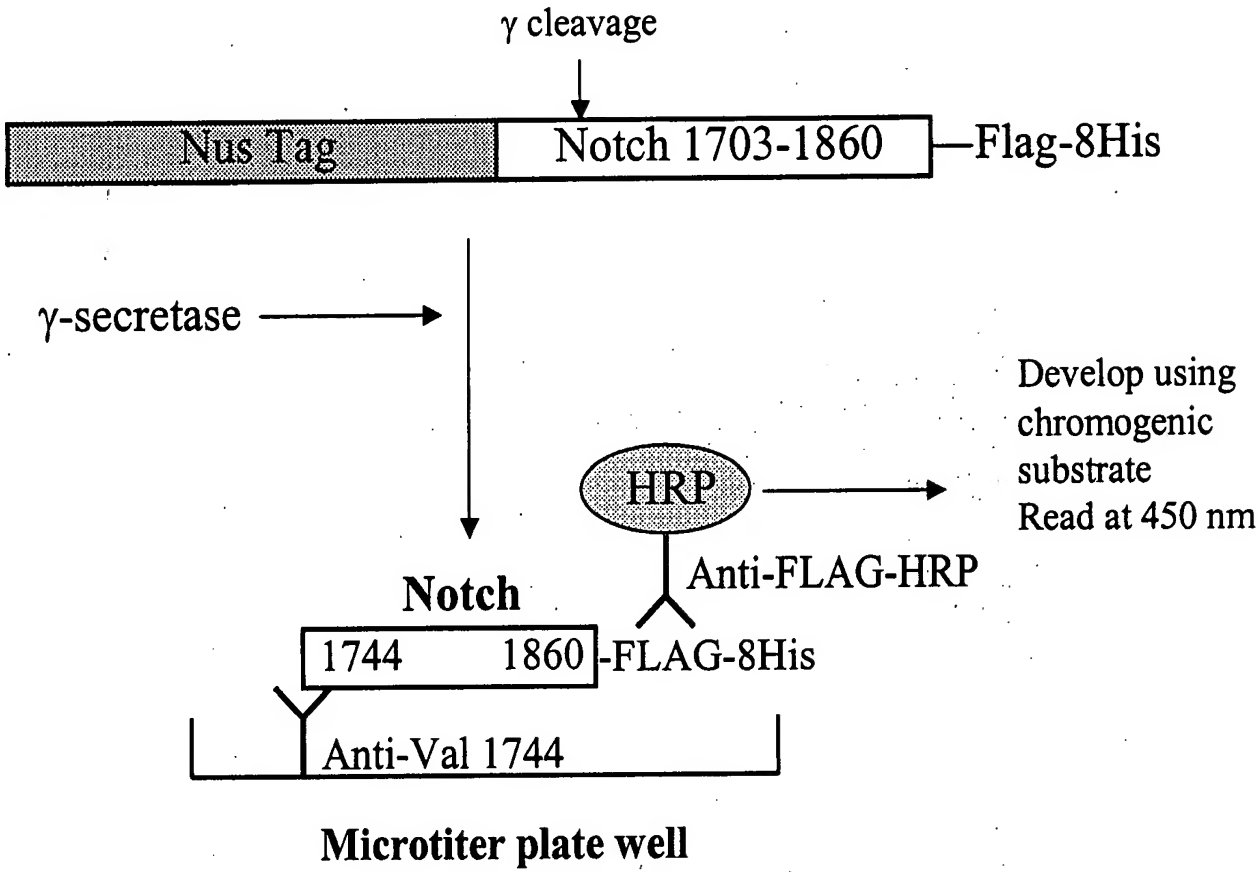
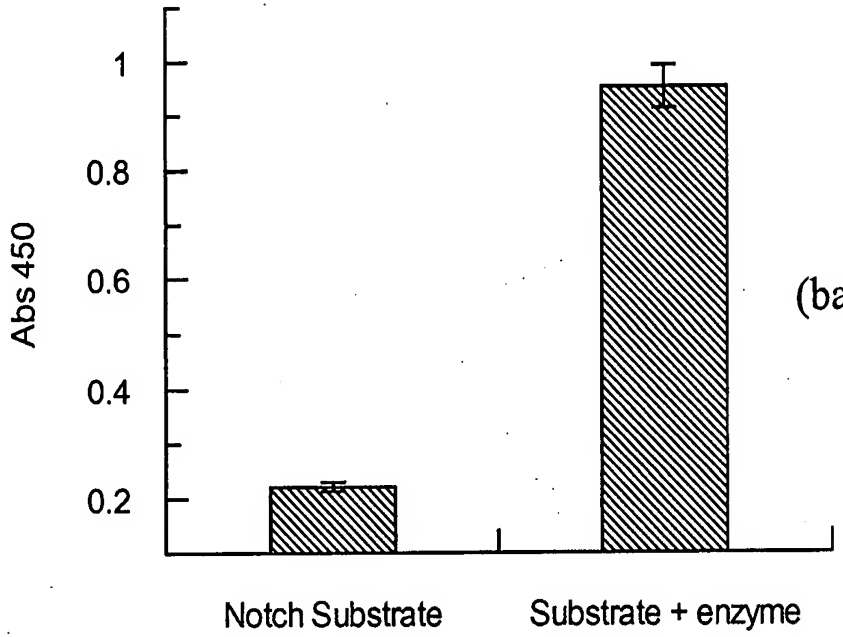

γ cleavage
Nus Tag
Notch 1703-1860
—Flag-8His
γ-secretase
Develop using
chromogenic
substrate
Read at 450 nm
HRP
Anti-FLAG-HRP
Notch
1744
1860
-FLAG-8His
Anti-Val 1744
Microtiter plate well

FIG. 7



Approx. 5-fold signal:background
(background using enzyme alone is negligible)

FIG. 8

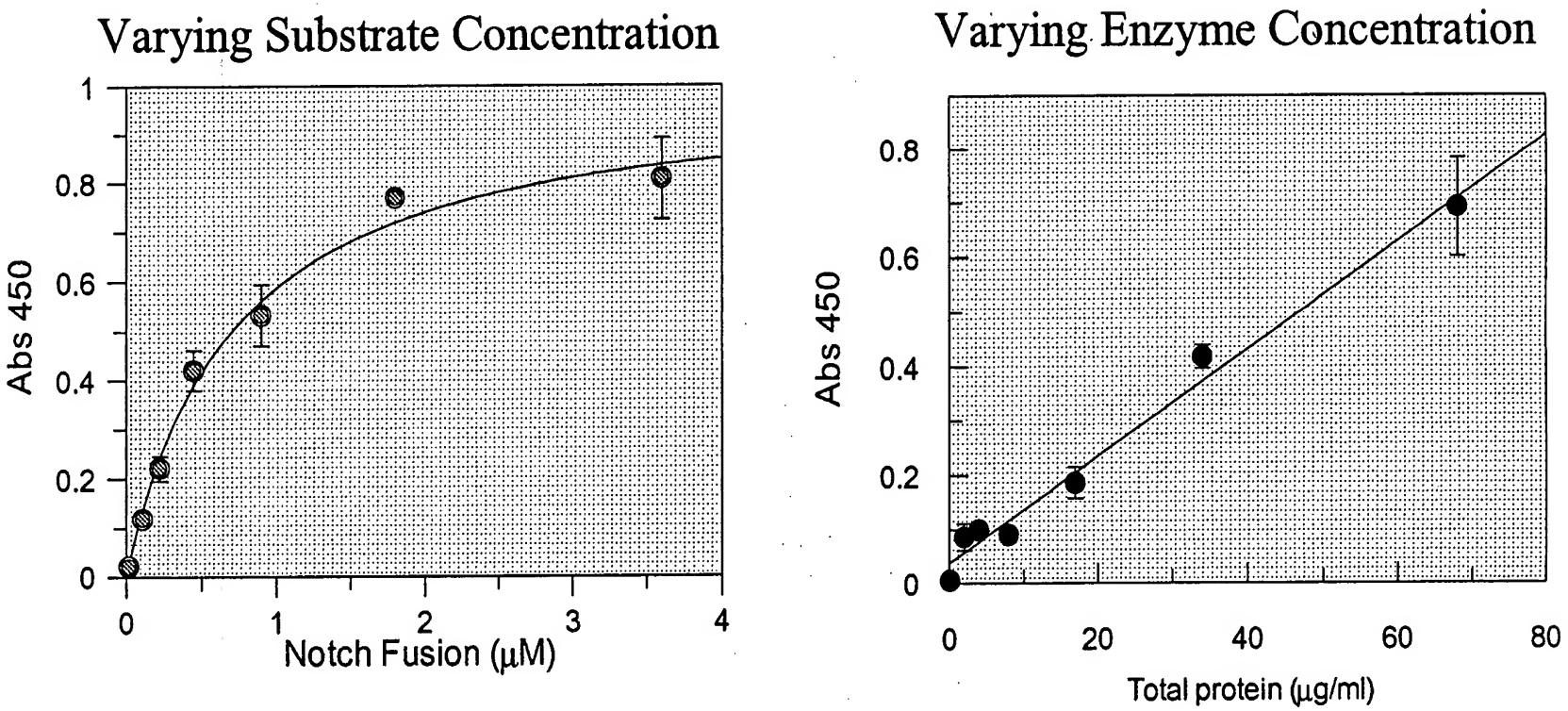
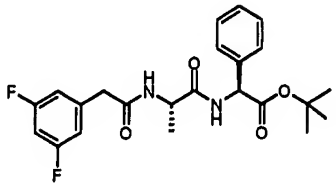
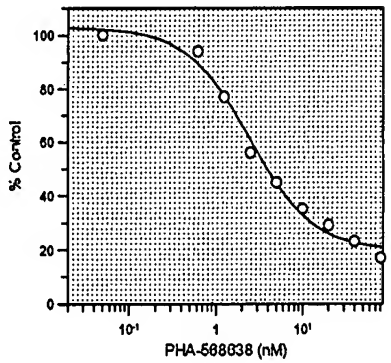

Varying Substrate Concentration
Abs 450
1
0.8
0.6
0.4
0.2
0
0
1
2
3
4
Notch Fusion (μM)
Varying Enzyme Concentration
Abs 450
0.8
0.6
0.4
0.2
0
0
20
40
60
80
Total protein (μg/ml)

FIG. 9

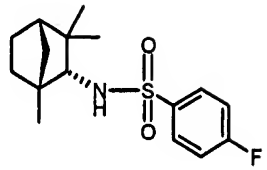
DAPT
(PHA-568638)

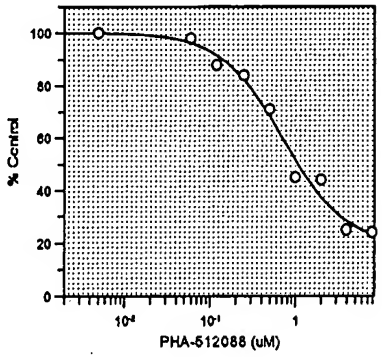




IC_{50} = 2.4 nM

Fenchylamine
(PHA-512088)





IC_{50} = 0.7 μM